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Studies on some interactions between potatoes and *Phytophthora infestans*

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The initial phases of host–parasite interactions have been studied by exposing zoospores of *Phytophthora infestans* to stem fragments of miniaturized potatoes. The compatibility or incompatibility of the interaction was reflected by the presence or absence of host colonization.

Observations began at the onset of incubation and showed that the duration of the zoospore mobility phase, the positions of encystment and the capacity for germination all seemed to be related to the compatibility or incompatibility of the interacting organisms. The immediate reaction of zoospores to the presence of the host indicated that the parasite was sensitive to substances formed by the plant before infection. These substances could perturb the normal development of an initially compatible combination.

These initial interactions were followed by an inducible defence mechanism which appeared 22 h after an initial contact with zoospores of an incompatible race or with a zoospore homogenate. This mechanism confered a long-lasting protection of plant fragments against new infection.

Two levels of control of the interactions between potato plants and *Phytophthora infestans* are discussed.

INTRODUCTION

Potatoes possess two distinct, genetically determined, mechanisms of resistance to *Phytophthora infestans* [*26*]. One mechanism is non-specific and is governed by a relatively large number of genes. It creates barriers or physiological conditions which are unfavourable for the development of an infection; it is not specifically directed against a particular pathogen. The other mechanism can be demonstrated during various challenges between *P. infestans* isolates and the progeny of crosses between *Solanum tuberosum* and *S. demissum.* These challenges lead to only two reactions, the first of which is an incompatibility reaction. Host cells degenerate

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immediately after contact with the fungus and die while proximal, healthy tissues react by producing fungitoxins which arrest parasite progression. Compatibility is reflected by the progressive colonization of the host by the parasite. These two interactions are controlled by the major plant resistance genes and parasite virulence genes [*3*].

Incompatibility is marked by an increased synthesis of fungitoxic terpene phytoalexins by the host. Among these phytoalexins, rishitin [*25*] and phytuberin [*28, 29*] have been extensively studied. Various stimuli cause the accumulation of these substances [*16*], and optimal concentrations are reached only 24 to 48 h after inoculation, as a function of the varieties used [*22*].

The relatively late accumulation of phytoalexins provoked by non-specific agents suggests that these compounds have a delayed rôle in the relationships between potatoes and *P. infestans*, whereas the gene-for-gene relationships [*17*], demonstrated by genetic analysis and pathogenicity tests, imply that probably there are earlier recognition signals between the two partners [*16, 27*].

Until the present time, potato–*P. infestans* interactions have been studied primarily with tuber slices or with petioles and laminae of leaves. With these systems, incompatibility is detected as a browning of the inoculated zone, the quantitation of necrotic cells or the chemical detection of phytoalexins, i.e. by delayed reactions. The present report describes a system of challenge *in vitro* using axenic and dwarf plantlets [*15*]. It has enabled us to observe early manifestations of compatibility and incompatibility which are probably more directly related to the recognition signals of the two organisms.

MATERIALS AND METHODS

Potatoes

The BF 15 and Houma cultivars have no major resistance gene and are sensitive to all strains of *P. infestans*. The varieties Black 1, Kennebec and Merimac have gene *R1*, which confers resistence to all pathotypes which do not have virulence *1*. Black 4 plants contain gene *R4* and are sensitive to only the fungal strain containing virulence *4*.

Houma, Kennebec and Merimac were provided by Dr Zacharius of the U.S. Department of Agriculture, Pennsylvania. Black 1 and Black 4 were obtained from Dr Madec of the C.N.R.A., Landernau, France. These varieties were used in the form of axenic dwarf plantlets, supplied by the laboratory of Professor Nozeran, Université de Paris Sud, Orsay, France. They were obtained by successive transfers of newly formed plants in 2·5 × 25 cm test tubes containing 80 ml of an agar solution of modified Knop medium [*19*]. The plantlets were obtained from cuttings initially derived from tuber buds.

Growth chambers were set at 19 °C with 12 000 to 13 000 lx of "industrial white" fluorescent light for 12 h per day. Under these conditions, plantlets arising from one-node plantings reached a length of 20 cm with 10 to 15 internodes after a mean period of 45 days.

Modified Knop medium contained the following: 2·12 mM $Ca(NO_3)_2.4H_2O$; 0·92 mM KH_2PO_4; 0·5 mM $MgSO_4.7H_2O$; 6·2 mM KNO_3; 12 g of Difco Agar and 58·5 mM sucrose, 10 ml of a micro-element solution, 10 ml of a ferric iron solution and

1 l of double distilled water. The micro-element solution was obtained by diluting 50 ml of the following solution to 1 l: 11·8 mM $MnSO_4.H_2O$; 3 mM KI; 0·2 mM $NiCl_2.6H_2O$; 0·2 mM $CoCl_2.6H_20$; 0·8 mM $TiO_2SO_3.5H_2O$; 0·35 mM $ZnSO_4.7H_2O$; 0·2 mM $CuSO_4.5H_2O$; 0·56 mM $BeSO_4.4H_2O$; 0·8 mM H_3BO_3, 9 mM H_2SO_4 and 1 l of double distilled water. The iron salt solution was obtained by diluting 102 mM $Fe_2(SO_4)_3.5H_2O$, clarified with 9 mM H_2SO_4, to 1 l.

Phytophthora infestans

Strains *0*, *1* and *4* were supplied by Dr Zacharius of the U.S.D.A. The parasite was cultured on an agar-containing medium with 100 g of greenpeas per l and 50 g of chick-peas per l, macerated for 2 h at 80 °C. The incubation was in a growth chamber in darkness at 20 °C and 60% relative humidity. Ten cm diameter agar plates were covered by white fungal mycelium 8 days after inoculation with an 8 mm diameter inoculum. The three pathotypes sporulated after 8 to 15 days. The sporangia were harvested by depositing 5 ml of distilled water on the mycelium, which was then gently scraped with a colony spreader. The sporangia were washed 3 times with sterile distilled water and were maintained in suspension for 2 h at 10 °C. Release of zoospores was obtained by raising the temperature to 20 °C. The motility phase in distilled water lasted for 90 to 120 min. and was followed by arrest, encystment and germination.

Although these standard conditions have been used by many authors, they nonetheless yield variable results concerning zoosporocyst production, zoospore liberation and germination [*30*].

After germination, all the encysted zoospores form a hypha which reaches about 50 μm in distilled water or in normal culture media; however, 95% of them do not ramify and cease to grow [*6*].

Challenges

Challenges were performed in 50 mm diameter Petri dishes containing modified solid Knop medium. Internode fragments of 10 to 15 mm long plantlets, excluding the apex, nodes and collar, were placed in the medium, followed by 0·05 ml of a suspension containing $5 \cdot 10^4$ zoospores per ml. Challenges were examined immediately using low power magnification, or were examined after 4 to 8 days of incubation at 20 °C in darkness. Each experimental and control treatment involved at least five replicates. With this system of challenge *in vitro*, the various factors can be rigidly controlled. Reproducibility is excellent within certain limits of quantity and quality of inoculum and of the vigour and physiology of the plantlets. If the density of the inoculum is lower than 10^2, then compatibility is not expressed, but if the density is greater than 10^5 zoospores, compatibility and incompatibility are confused. Similarly, plantlets which are too old, chlorotic or have been induced to form tubers [*10*], lead to incompatible reactions which are less distinct and occasionally annulled.

Preparation of plantlet extracts

Portions of 10 to 15 mm long stems arising from segments of two plantlets were incubated for 24 h in 2 ml of sterile water at 20 °C. The fragments were then removed and the solution was used immediately.

Preparation of zoospore homogenates

Twenty ml of a suspension containing 10^6 zoospores per ml were placed in a flask containing 20 g of 0·55 mm diameter glass beads. The flask was placed in a Braun Type 2876 glass bead homogenizer and chilled with a CO_2 stream. The flask was prechilled for 2 s and agitated at low speed for 20 s. It was chilled again for 2 s and agitated for a total of 60 s, with 2 s of chilling every 10 s. The crude homogenate was used immediately.

RESULTS

Immediate observations of plantlet–zoospore challenges in vitro

Microscopic observations performed immediately after exposure showed that the behaviour of the zoospores differed in compatible and incompatible combinations. These differences involved the length of the zoospore motility phase, the positions of encystment and the capacity to form a germination hypha. The last observation was performed after 24 h at 20 °C in darkness. In this series of observations we studied compatible combinations consisting of: Black 1 (*R1*), Kennebec (*R1*) or Merimac (*R1*), containing the major resistance gene *R1* and race *1* of the fungus; or Black 4 (*R4*) and race *4*. Incompatible combinations consisted of Kennebec (*R1*), Merimac (*R1*) or Black 1 (*R1*) and race *4*; or Black 4 (*R4*) challenged with pathotype 1. The results were as follows.

Length of the zoospore motility phase. The zoospore population was heterogenous concerning the exact moment of their emission from sporangia. Some were liberated during the 10 °C maturation period, while others emerged only after the return to 20 °C. Consequently, it is difficult to evaluate precisely the length of the motility phase for the entire zoospore population. We could, nonetheless, observe that zoospores remained motile for 45 to 60 min in Knop medium in the absence of the host, i.e. far less time than in distilled water (90 to 120 min). In the presence of a fragment of compatible stem, motility lasted for 15 to 20 min, while zoospores were immobilized and encysted in less than 5 min when the fragment was from an incompatible variety.

Encystment positions. When zoospores were present alone on the agar substrate they swam with no apparent tropism and then encysted at random within the limits of the drop. After an incubation for 24 h at 20 °C, the vast majority formed a germ tube. During the short motility phase of an incompatible challenge, the zoospores tended to move away from the potato fragment and encysted at some distance from it. Their capacity to produce a germ tube was reduced, and most had not evolved after 24 h (Plate 1). It should be noted, however, that certain germinations were efficient, since degenerating and necrotic intercellular hyphae were visible in the first two or three cortical parenchyma layers of the stem 72 h after inoculation (Caporali & Currey, personal communication).

The zoospores of a compatible combination formed a fringe at 1 mm from the edge of the plant fragment, encysted and then normally formed a germ tube. After 24 h, certain germ tubes had continued their development and had reached the host (Plate 2).

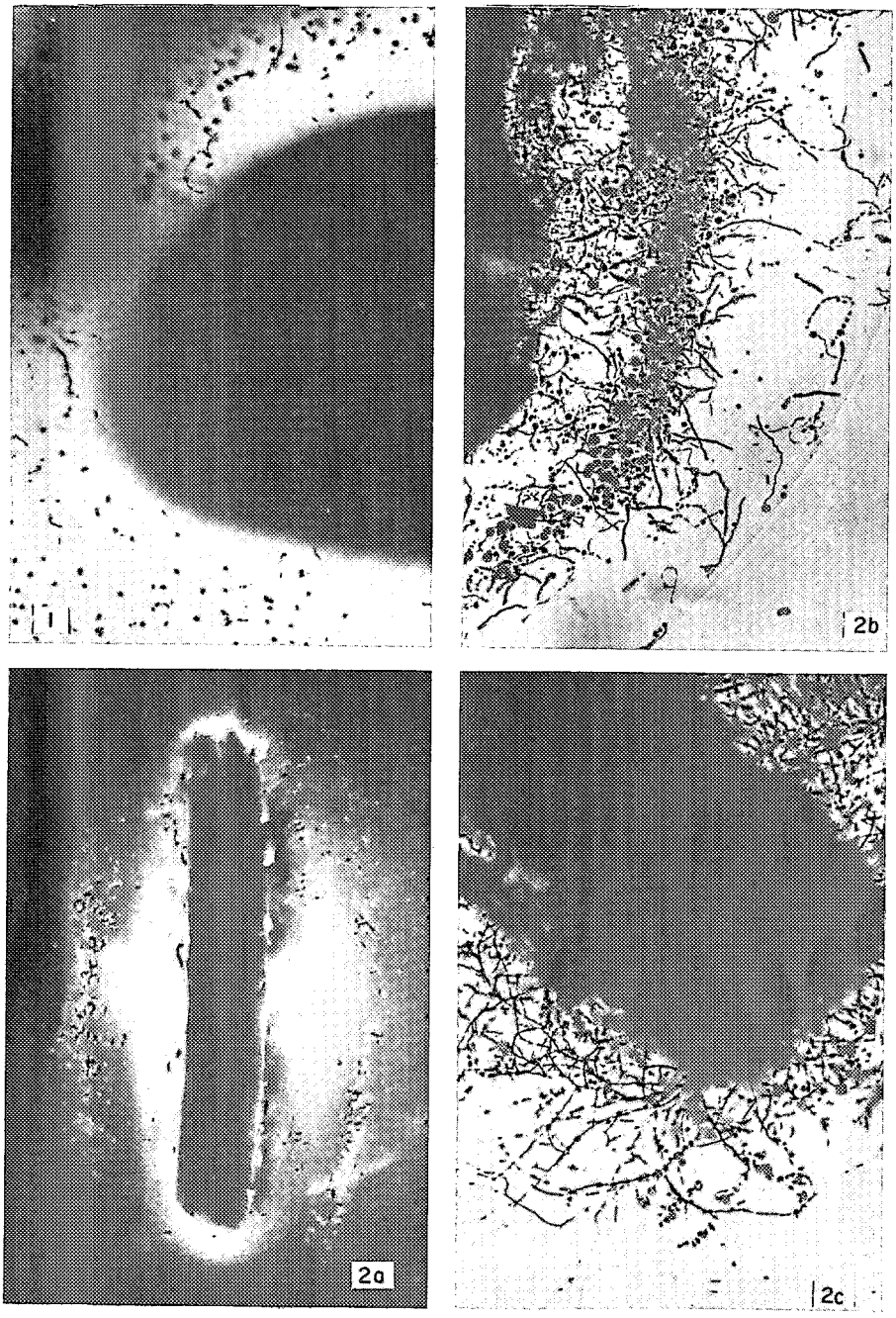

PLATE 1. Incompatible challenge [Kennebec (*R1*)—race 4] observed 24 h after the deposition of zoospores.

PLATE 2. Compatible challenge [Kennebec (*R1*)—race 1] observed 1 h (a, × 8), 24 h (b, × 40) and 102 h (c, × 50) after deposition of zoospores. The zoospores are first grouped in a fringe. Encysted cells germinate, followed by colonization of the stem fragment by the mycelium which differentiates new zoosporocysts.

[*facing page 4*]

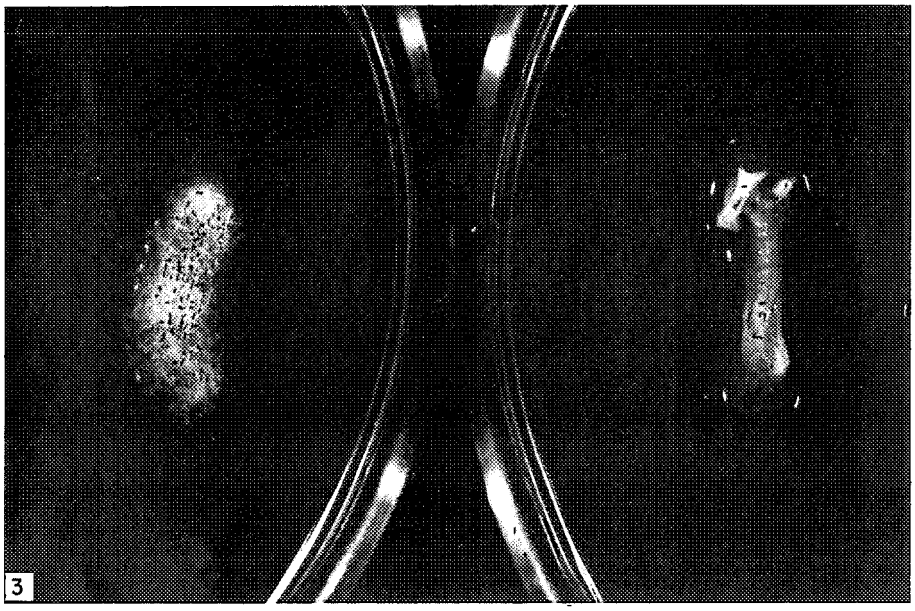

PLATE 3. Incubation of stem fragments and zoospores. Observation after 8 days at 20 °C (×8). Left: compatible combination [Merimac (*R1*) infected by race 1] Right: incompatible combination [Merimac (*R*1) infected by race 4].

PLATE 4. Sensitivity of race 1 to substances leached from varieties Black 4 (*R4*) (left) and Kennebec (*R1*) (right). These observations were made after 24 h of incubation in the presence of the exudates.

Later observations of fungus–plantlet challenges in vitro

Macroscopic observations performed 7 to 10 days after inoculation clearly demonstrated the compatible or incompatible nature of the combination. In cases of incompatibility, the fungus had not proliferated and the plant fragment was apparently untouched. Fragments of compatible combinations, however, were covered with a white mycelial mat, visible with the naked eye, resulting from the colonization of the host tissue by the *P. infestans* hyphae (Plate 3). These hyphae developed on the stem and could be detected as soon as 4 days after inoculation. They were abundant and were covered with numerous newly formed zoosporocysts 8 days after inoculation.

Demonstration of substances synthesized by the host before infection

The existence of substances secreted by the plant is implied by the reaction of all the zoospores in the inoculum to the host. Two experiments were made to determine whether these substances were synthesized before or after contact with the pathogen.

In the first experiment, the variations in germination capacity of zoospores in the presence or absence of host substances was investigated. Difficulties in this type of experiment arise from spontaneous fluctuations of germination in controls.

We observed variations of germination in distilled water in the range of 0 to 95%. These variations may be attributed to several factors. The nutritional medium was based on commercially supplied greenpeas and chickpeas, whose quality fluctuates with different lots. Other factors, such as the age of the culture from which sporangia were obtained, bacterial contamination and the time which elapsed between emission and utilization of the zoospores may also have an influence (Legrand, personal communication), but these factors can be fairly easily controlled. In this report, we present only the results of those experiments in which controls demonstrated the satisfactory physiological state of the *P. infestans* strain employed. In addition, figures for percentage germination of zoospores in various experimental situations represents the capacity of the zoospore to produce a germ and not its capacity to form hyphae which would colonize a host plant subsequently.

Fragments of stems from Kennebec (*R1*) and Black 4 (*R4*), 10 to 15 mm long, were incubated for 24 h at 20 °C in a drop of sterile distilled water on the surface of solid Knop medium. The plant fragments were then removed and 0·05 ml of a suspension of either race 1 or race 4 zoospores were added in order to obtain two compatible combinations (*R1* exudate—race 1; and *R4* exudate—race 4) and two incompatible combinations (*R1* exudate—race 4; and *R4* exudate—race 1). Regardless of the combination, zoospores swam for 15 to 20 min with no apparent tropism and then encysted at random. After 24 h at 20 °C, there were differences in the capacities of the zoospores to produce germ tubes (Table 1, Plate 4). The percentage of zoospores having formed a germ tube after 24 h (three experiments) is shown in Table 1. Each experiment involved a different exudate, but zoospores from the same culture and 10 to 12 independent microscope fields were observed in each. The homogeneity of the replicates used to calculate the percentages was verified with the χ^2 tests. The normal law was used to calculate the 5% confidence limits. The results obtained with both fungal faces were shown to be significantly different by performing

TABLE 1

Demonstration of substances synthesized by the potato plant before inoculation: action of diffusates on production of germ tubes in vitro *by races 1 and 4. (Germination was scored after 24 h of incubation at 20 °C. Each experiment involved an exudate from a different plantlet)*

	Control (distilled water)					Race I Compatible diffusate (variety *R1*)					Incompatible diffusate (variety *R4*)				
					Total					Total					Total
Germinated	57	36	65	80	238	47	44	34	125		31	44	56	131	
Non-germinated	5	6	11	17	39	60	65	34	159		89	71	111	271	
Total	62	42	76	97	277	107	109	68	284		120	115	167	402	
Homogeneity test	$\chi^2 = 1{\cdot}35$					$\chi^2 = 1{\cdot}58$					$\chi^2 = 4{\cdot}24$				
Comparison test	$238/277 = 85{\cdot}9 \pm 4{\cdot}1$					$125/284 = 44 \pm 6{\cdot}2$					$131/402 = 32{\cdot}6 \pm 4{\cdot}5$				
	$\chi^2 = 188$					$\chi^2 = 9{\cdot}29$									
	$\chi^2 = 108$														

	Control (distilled water)							Race IV Incompatible diffusate (variety *R1*)							Compatible diffusate (variety *R4*)					
							Total						Total							Total
Germinated	87	124	86	112	164	19	592	24	21	32	19	96		26	34	33	93			
Non-germinated	15	19	16	27	18	6	101	259	195	218	108	780		92	94	183	369			
Total	102	143	102	139	182	25	693	283	216	250	127	876		118	128	216	462			
Homogeniety test	$\chi^2 = 7{\cdot}91$							$\chi^2 = 5{\cdot}07$							$\chi^2 = 6{\cdot}72$					
Comparison test	$592/693 = 85{\cdot}4 \pm 4{\cdot}6$							$96/876 = 10{\cdot}95 \pm 2{\cdot}05$							$93/462 = 20{\cdot}1 \pm 2{\cdot}3$					
	$\chi^2 = 1000$							$\chi^2 = 21$												
	$\chi^2 = 490$																			

2×2 comparisons of the percentages of germinated spores in each experiment with the χ^2 test at the 1% threshold.

The germination of zoospores thus appeared to be influenced by a potato plantlet exudate formed without contact with the parasite. This effect was greater when the exudate was from an incompatible variety.

The second series of experiments involved the incubation of 20 stem fragments (10 to 15 mm long) in 2 ml of sterile distilled water for 24 h at 20 °C. The plant material was then discarded. The diffusates obtained from Houma (no major resistance gene to *P. infestans*), Kennebec (*R1*) and Black 4 (*R4*) were used to supplement compatible and incompatible combinations composed of these three varieties and fungal races 1 and 4. Compatible and incompatible controls received only distilled water. The presence or absence of host colonization was noted after 8 days of incubation at 20 °C. Three experiments were performed, each involving five replicates. The result of two experiments are shown in Table 2.

It can be seen that, with only two exceptions, combinations having received tissue diffusate were similar to the controls which had received distilled water. The

TABLE 2

Demonstration of substances synthesized by the potato plant before inoculation: action of diffusates on the evolution of compatible and incompatible combinations. [*Compatibility and incompatibility were noted, respectively, by the colonization* (+) *or the non-colonization* (−) *of the host*]

Couples	Blank no diffusate	Houma diffusate (*r*)	Kennebec diffusate (*R1*)	Black 4 diffusate (*R4*)
Houma *r*/Race 1	+ Compatible	+	+	+
Houma *r*/Race 4	+ Compatible	+	+	+
Kennebec *R1*/Race 1	+ Compatible	+	+	−
Kennebec *R1*/Race 4	− Incompatible	−	−	−
Black 4 *R4*/Race 1	− Incompatible	−	−	−
Black 4 *R4*/Race 4	+ Compatible	+	−	+

two exceptions involved the action of an incompatible diffusate (in comparison to the infecting race of the fungus) on a compatible zoospore–plantlet combination: instead of being colonized, as expected, the plant fragments were free of mycelial infestation. This protection was acquired only by Kennebec (*R1*) and Black 4 (*R4*), which have at least one resistance gene R, but was not acquired by Houma, which has none.

The third series of experiments was performed with slightly senescent plants. In comparison to the two experiments presented above, there were no notable differences between treatments after 8 days. Protection seemed to be acquired by compatible varieties containing an *R* gene and supplemented with diffusate from an incompatible host. If, however, the observations were extended to 12 and 15 days post-inoculation, we could observe less intense and more delayed mycelial development than on controls on plantlet fragments having received the incompatible diffusate. Although protection was not absolute in this third series of experiments, it nevertheless should be noted that the addition of a diffusate, from an incomplete variety formed in the absence of any contact with the parasite, could considerably delay and attenuate the development of infection in a compatible combination.

Thus, potato plantlets appear to excrete substances in the absence of any contact with the parasite which act on the capacity of encysted zoospores to produce germ tubes. They perturb the normal development of an initially compatible interaction, provided the receptor variety contains a major gene for resistance.

Demonstration of an inducible defence mechanism

The system of zoospore–plantlet challenge *in vitro* was used to demonstrate the existence of an inducible defence mechanism comparable to that shown in tubers [*14*, *29*] or in calluses [*12*, *13*, *20*].

Batches of plantlet fragments of Kennebec (*R1*) were pre-inoculated with the

TABLE 3

Delay in the induction of an efficient incompatible reaction protecting the potato plant from infection by a virulent pathotype. [*Plantlet fragments were first inoculated with an incompatible race and then by a compatible race. The resistance reaction was noted by the non-colonization* (−) *of the host*]

Incubation delay time (h) between two inoculations	Success (+ = colonization) or failure (− = no colonization) of the second inoculation by a compatible race
0	+
1	+
4	+
11	+
22	+
36	−
52	−
70	−
92	−

TABLE 4

Induction by a zoospore homogenate of an incompatible reaction in a potato variety without major resistance genes (compatible with all races of P. infestans). [*Compatibility and non-compatibility were noted, respectively, by the colonization* (+) *or the non-colonization* (−) *of the host*]

Variety	When infected		Infection successful (+) unsuccessful (−)
	Immediately	After 3 days	
Kennebec	Race 0		−
(*R1*)	Race 1		+
	Race 0	Race 1	−
	Race 0 + race 1		+
	Cell-free homogenate 0+ race 1		+
	Cell-free homogenate 0	Race 1	−
BF 5	Race 0		+
(*r*)	Race 1		+
	Cell-free homogenate 0+ race 1	——	+
	Cell-free homogenate 0	Race 1	−

incompatible race, virulence 0, and were post-inoculated after 1, 4, 11, 22, 36, 52, 70 and 92 h of incubation at 20 °C with a suspension of compatible pathotype 1 zoospores. All were incubated at 20 °C for 8 days and colonization of the fragments was then scored (Table 3). Batches which were left in contact with the incompatible race for less than 22 h were colonized, while those superinfected after 36 h did not support the development of mycelia. They thus acquired an incompatibility towards race 1, normally compatible, as a result of the protection induced by race 0,

A similar protection could be induced after similar delays by substituting for the incompatible race 0 zoospores a cell-free homogenate of the same race. In this case protection was extended to variety BF 15, which has no *R* gene and is in principle compatible with all races of *P. infectans* (Table 4).

DISCUSSION

The incubation of fragments of axenic potato plantlet stems and *P. infestans* zoospores *in vitro* led to the unambiguous and rapid determination of the compatibility or incompatibility of the combination formed after a simple visual observation. Zoospores reacted immediately and differentially to the presence of sensitive or resistant plantlets. In addition to enabling early observations to be performed, this system showed that plantlets also had an inducible defence which, as in tubers, could be triggered by an incompatible race of *P. infestans* or by a zoospore extract. This induced incompatibility was directed against all *P. infestans* races and was manifested after incubation by the absence of mycelial proliferation. This response of plantlets appeared after periods comparable to those observed for tubers or entire plants. It was also accompanied by the accumulation of rishitin (de Vallavieille & Nandris, unpublished results). Our challenge system could be easily adapted to continue the characterization of the triggering substance of the hypersensitive reaction begun by Currier; this substance is probably localized in the *P. infestans* zoospore membrane [*7*, *17*].

Our system has enabled us to demonstrate that the potato forms substances involved in pathogenesis and that their formation occurs in the absence of any contact with the parasite. These substances attenuate the capacity of encysted zoospores to produce germ tubes and are also capable of modifying a normally compatible interaction in combinations where the host contains at least one major resistance gene. We wish to compare this result with data in the literature concerning the study of interactions between potatoes and *P. infestans*.

In our challenge system, the germination capacity of zoospores was affected by substances emitted by the host. This observation is apparently contradictory to those of other workers [*8*, *20*, *24*] who suggested that, in compatible as well as in incompatible combinations, zoospores encysted on the surface of the host and germinated to form a hypha which differentiated an appressorium and then a filament which grew and ramified within the cells of the host. This contradiction is not apparent, since the inhibition of germination that we observed was never total; in addition, in fragments of incompatible plantlets, necrotic intercellular hyphae were observed in the superficial layers of the cortical parenchyma (Caporali & Curry, unpublished observations).

These studies of potato–*P. infestans* interactions which employ fragments of axenic plantlets confirm and extend the results obtained by Ingram *et al.* [*12*, *13*] with cell cultures *in vitro*, free or aggregated in calluses, arising either from tubers or from stems. With this challenge system, the authors showed that the resistance reaction was a tissue response and was not cellular and that it involved the participation of phenolic compounds formed in response to the infection [*21*]. They did not, however, demonstrate the existence of factors synthesized in the absence of contact with the parasite. This difference between the two challenge systems may be explained by the fact that one used tissue cultures with growing and partially differentiated cells which were removed from inter-tissue interaction systems, thus expressing only a fraction of their potentialities. Thus, the production of antibiotic by *Phyllanthus urinaria* tissue cultures is reduced and ultimately lost with successive transfers of calluses [*11*].

The existence of inhibitory compounds, preformed by the plant and involved in

pathogenesis, is well known (cf. [*23*] for review). Their action in the determination of parasite specificity, however, is most often at the level of different pathogenic species and may be explained by two distinct mechanisms. The compatible pathogenic organism may detoxify, e.g. it may secrete an enzyme which inactivates the preformed inhibitory substance which remains active against all pathogenic species not having this detoxifying activity [*1*]. Alternatively, the preformed molecule may be present in the plant in an inactive form which is converted to an active form during attack by enzymes present in potentially pathogenic micro-organisms [*14*]; only species lacking this enzyme would be parasitic. With the exception of peas [*4*] and tulips [*2*] there are few host–parasite systems known where components formed by the plant in the absence of any contact with the pathogen have a specificity in relation to the various physiological races of the same fungal species. The potato–*P. infestans* relationships obviously involve such substances, probably involved in recognition mechanisms. Their chemical characterization and the determination of their mode of action should enable us to more satisfactorily understand "gene-for-gene" interactions between the two organisms.

Axenic miniaturized potato plantlets possessed an inducible system of defence, as do tubers. This was triggered by an incomplete race of *P. infestans* or by a zoospore homogenate. These results confirm the work of Kiraly *et al.* [*14*] and Currier [*7*]. All *P. infestans* races have the inducer of this reaction but it is manifest only in incompatible challenges. Doke *et al.* [*5*] suggested the formation of a molecular complex between a fraction of a plant homogenate and one of a zoospore homogenate which could trigger the incompatibility reaction. In addition, Kitazawa *et al.* [*15*] performed microcinematography on petiole cells inoculated with an incompatible race and suggested the existence of interactions at the host–parasite interface between substances arising from infestation hyphae and compounds excreted by the cytoplasm of host cells. These observations are consistent with the hypothesis of an active participation in pathogenesis of compounds arising from the parasite in addition to substances preformed by the plant.

The relationships between the potato and *P. infestans* involves exchanges of information between the host plant and the parasitic fungus. The plant appears to have two potential defence mechanisms which are mobilized successively. The first is activated at the moment of contact with the parasite, which reacts specifically to substances preformed by the plant. The second is probably induced by the functioning of the first, although we do not have experimental evidence to support this hypothesis. It appears to create a state of "incompatibility" characterized by the necrosis of cells in contact with the parasite, the accumulation of fungitoxic terpenoids in proximal healthy tissues and by the arrest of the parastitic invasion. If the second mechanism is not induced, the pathogenic organism propagates freely in the compatible tissues of the host.

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RESUMEN

El contacto entre zoosporas y fragmentos de tallos de plantas de patata fenotipicamente miniaturizadas permite observar las faces iniciales de la interacción de la planta huésped y el hongo parásito. El funcionamiento de los genes mayores de resistencia de la patata y de los genes de virulencia del parásito

determina la compatibilidad o la incompatibilidad entre huésped y parásito: colonización o no colonización del huesped.

Las observaciones, efectuadas, al comienzo del contacto, indican que el tiempo de la face de motilitad del parásito, las posiciones del enquistamiento o y la aptitud a germinar, dependen de la compatibilidad o de la incompatibilidad de la pareja huésped–parásito.

La reacción immediata de las zoosporas al contacto con la planta huésped, muestra la presencia en la patata de substancias synthetizas antes de la contaminación.

Estas substancias podrian perturbar el desarrollo normal de un contacto inicialmente compatible.

A este tipo de interacción inicial, continua un mecanismo de defenza inductible, mecanismo que aparece a las 22 hs, luego del primer contacto con el parásito de une raza incompatible o con el homogenado de la zoospora. En estas condiciones el fragmento de planta esta protegido de una nueva infección. La existencia en, la patata, de dos niveles de control con respecto a su interaccion con el hongo parásito será discutido.

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